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Mounting techniques for the preservation and analysis of diatoms

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INTRODUCTION

In order that diatoms can be safely stored and preserved, they are mounted and displayed on microscope slides. These slides are curated to provide a scientific, taxonomic, and historical reference collection. The method presented here will provide new workers with a comprehensive schedule for the preparation of consistent, good quality slides.

EQUIPMENT

The only specialized equipment required is a low power, wide field dissection microscope and a small revolving table for ringing coverslips. Ideally the microscope should be fitted with forearm rests which allow total freedom of wrist and hand movement. All other pieces of equipment can be cheaply acquired or handmade.

Above all the operator should be comfortable and all the equipment arranged logically within easy reach. The working area should be well lit, clean, and free from draughts and distraction; on occasion difficult mounts require great concentration and patience.

METHOD

The preparation, mounting, and finishing steps are laid out below.

Slide preparation

1. Take a new microscope slide and with a moist tissue dipped in scouring powder gently abrade one surface. (This keys the glass surface ready for mounting.) Thoroughly clean the slide in warm soapy water to remove the scouring powder and any grease, fingerprints, etc. Rinse the slide in distilled water and store under ethanol spirit.
2. Polish a clean coverslip (No. 0. 13 mm diameter) with a lens tissue or fibre-free cloth.
3. Place a small drop of distilled water on the centre of a revolving table and place the coverslip onto the water. A sufficiently small drop of water will spread under the coverslip and cause it to adhere to the revolving surface. Carefully centralize the coverslip on the table.
4. Trim a fine paint brush to a point and using a quality Indian ink, scribe a circle of the required size. To do this, gently spin the coverslip and touch the glass surface at one point with the tip of the paint brush lightly loaded with ink. Practice will enable rings of the desired size and thickness to be made. Put the prepared coverslip to one side to dry. If required, batches of slides and coverslips can be prepared prior to a mounting session.

Mounting

5. Take a prepared slide, leave to air dry and wipe with a lens tissue. Using a marked template, spot the centre of the unscoured slide surface with a fibre tipped pen.
6. Using a fine paint brush, apply as thin a smear of mountant glue as possible to the abraded surface. Gentle sweeping of the glue over the spotted area with the brush will remove any dust, airborne or static contamination.
7. Using a pig's eyelash or finely drawn glass fibre, pick up the diatom(s) and with gentle manipulation mount in the desired orientation over the ink spot. The viscosity of the glue will allow several mounted diatoms to be gently pushed together in a discrete area for comparative viewing, even under eventual high power microscopic examination.
8. Gently waft the slide over a low spirit lamp flame. The glue evaporates and hardens to a transparent film invisible under microscopic analysis. This will secure the diatoms as orientated during the rest of the slide preparation.
9. Take a prepared coverslip and with the ringed surface uppermost place a drop of mountant in the centre. Invert the coverslip and gently position the ringed area above the ink spot on the slide. Alternatively the mountant may be applied to the slide surface. Allow the mountant to spread under the coverslip as it settles under its own weight, guiding it into position if necessary. Do not press the coverslip down under any circumstances — damage to the diatom is guaranteed!

Finishing steps

10. Where time is not a limiting factor, the slide may now be placed in a hot 60°C oven and left to cure. For exceptionally fragile specimens or precious material this is probably the only safe way of making a slide. Gentle hardening of the mountant removes the need for further processing until the specimen is safely preserved. It takes about a week to cure a slide completely.
11. In many cases enclosed frustules trap air during mounting, which causes considerable aberration of detail under microscopic examination. To remove trapped air bubbles and considerably speed up the slide making process, the mountant can be rapidly hardened by cooking the slide. After step

9, gently warm the slide by wafting over a low spirit flame. This gentle warming reduces the viscosity of the mountant and the spirit diluent bubbles out of solution, rapidly curing the mountant. At the same time the trapped air is normally forced out of the specimen, replaced by the less viscous mountant. Any small air pockets which remain frequently shrink and disappear as the slide cools. Persistent air pockets may require several warming and cooling cycles. Gently tapping the slide on a hard surface may dislodge 'stuck' bubbles, but beware, this has the additional risk of dislodging the glued specimen(s), or in extreme cases, causing actual physical damage.

12. Once the slide has cooled to room temperature and the mountant is fully cured, any excess mountant which has bubbled out around the edges of the coverslip may be removed. This is done firstly, by gentle chipping and scraping with a pointed scalpel, and then by wiping away any final traces with a toluene soaked wipe.

13. Polish the slide carefully, removing any remaining ink spot under the slide. Do not press hard on the coverslip surface as the diatoms may break under the pressure.

14. The final step in the process is to examine critically the mounted specimen(s) under a high magnification. If the slide is acceptable then it should be labelled with as much information as possible. The Natural History Museum has purpose printed labels which are gummed to the slide and where available, the following information is written in ink using a very fine mapping pen: the date of mounting, the mounter's name or initials, the type of mountant used, a specimen identification (if known), the locality or collection site, a core number (if applicable), date of collection, and collector's name. The annotated slide labels are stuck to the slide and orientated such that they can be read in the slide trays without needing to be removed.

MOUNTANTS

In the past a succession of mountants have found favour with slide makers. Their use today is restricted on the grounds of health and safety. The properties of a good mountant are that it should be safe, indefinitely stable, and totally transparent when cured. The mountant should be readily available, and have a viscosity which may be altered to a required consistency. The mountant which fulfills these criteria and is used consistently in the diatom section at The Natural History Museum is Naphrax (N.B.S., 3 Betts Avenue, Martlesham Heath, Ipswich). Naphrax is supplied as a ready-to-use liquid diluted in toluene. Naphrax may be too fluid for some mounts as supplied, but a small sample left in a warm oven will increase in viscosity. Conversely, after a while the mountant may become too thick; dilution with toluene will remedy the problem.

MOUNTING GLUE

The glue used (step 6) should be totally transparent when dried, and of a viscosity that allows diatoms to be manipulated during the positioning part of the slide making procedure. Once in position the glue should be such that it may be dried quickly, ensuring the secure positioning and orientation of the specimens during the rest of the slide preparation. The glue used in the diatom section is prepared in the following way. A saturated solution of gum tragacanth in sterile distilled water is prepared and left to stand for at least a week in an airtight container. The clear supernatant layer is removed, and glycerol 10% w/v added. To prevent microbial growth a few crystals of phenol may be added or an anti-fouling agent. It is only neccessary to prepare a small amount of glue at a time; 10 mls can last as long as a year with constant use.

ADDITIONAL NOTES

More specialized techniques for the mounting of diatoms require a certain amount of manual dexterity, especially where large numbers of specimens are mounted on a single slide. Coverslips or slides etched with grids remove the need for ringing as an aid to specimen location.

Mounting selected diatoms on the coverslip, instead of on the slide, is sometimes preferred, as this allows precise positioning within the ringed area. The diatoms are presented fractionally nearer the microscope lens and in some instances this can afford greater image resolution under high power examination. The potential drawback to this is that if the glue is not applied thinly enough then puddling occurs at the point of diatom adherence and considerable aberration of the image results. Many specimens are very fragile and/or have thin delicate silica processes and when mounted on thin edges will smash under the weight of a settling coverslip. To help prevent this, small pieces of broken coverslip or sponge spicules may be glued around the coverslip edge to support the weight during slide making.

Sometimes the mountant will shrink during the curing process; also, where insufficient mountant has been used air pockets form. This in itself may not interfere with microscopic examination, but if the whole undersurface of the coverslip is not evenly supported the coverslip will flex, with an increased tendency for it to crack, especially during slide cleaning. In the event of such a problem occurring, it is possible to save the slide by placing a small drop of mountant close to the coverslip near the air pocket and gently warming the slide. The mountant will be drawn under the coverslip and should displace any air pockets. If a coverslip is broken and the specimen needs to be saved it is possible to repair a slide. Place the whole slide in a container and cover it in neat mountant diluent. The mountant will become soft and eventually melt, dissolving sufficiently to allow the coverslip to be gently lifted free. The specimen may remain glued to the slide, in which case remount with a fresh coverslip. In some cases, the glue will dissolve and the diatom should be recovered, washed in diluent and further washed in a descending alcohol series dilution into water, then dried and the mounting procedure repeated.

When the diatom frustule is composed of very fine pores, the viscosity of the mountant, even during warming, will not allow it to penetrate the diatom. Thinning the mountant should overcome the problem, but extra mountant to account for the additional spirit will need to be applied. Alternatively add a drop of spirit directly to the specimen on the slide, which will enable the mountant to penetrate the specimen more easily.

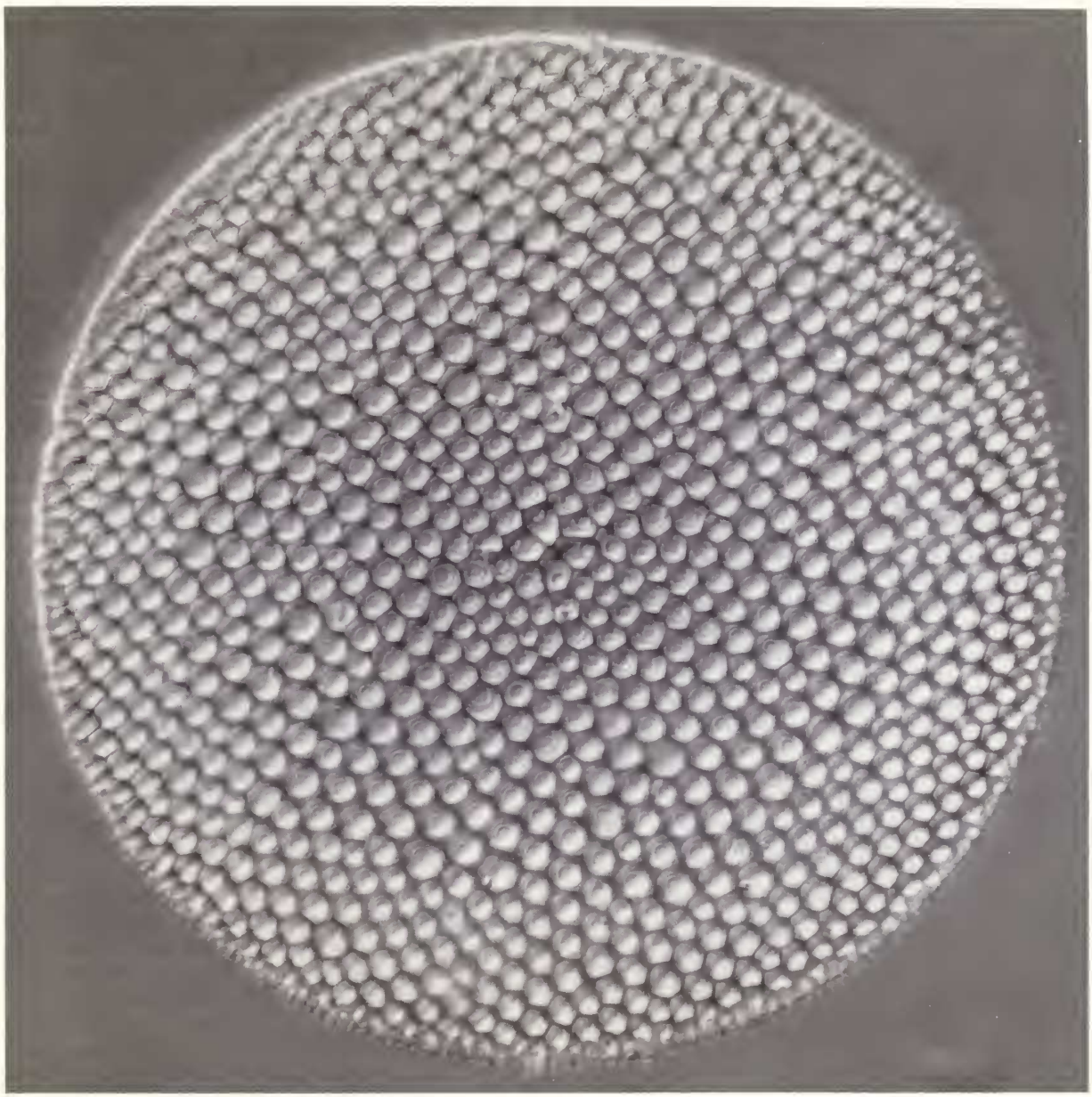

Fig.1. A Coscinodiscus fossil diatom slide, photographed using differential interference contrast photomicrography under high magnification (x1600). This demonstrates the high resolution of surface detail possible using the mounting technique described.

At all times when preparing slides be aware of the potential volatility of the mountant and diluent, especially when using naked flames. Potential fire risk may be avoided by having small volumes in tightly sealed bottles at the work station and bulk supplies suitably stored elsewhere. When curing slides take care not to boil the mountant excessively, as this will uncontrollably bounce the coverslip and smash the specimen. Extreme cases of boiling, or where dilute mountant is used can cause the evacuated vapour to flash ignite. Although this is not in itself dangerous (the amount of mountant present is too small), a surprised technician may drop the slide. Avoid placing hot slides on cold surfaces after curing as they will crack. Patient waiting until the slide cools is best, but bridging the slide across two wooden blocks will free the hands.

STREWN SLIDES

An alternative to individually mounted specimens, particularly for routine sample analysis (after sample cleaning, for example) or population monitoring, is that of strewn mounting. The basic slide making technique is similar to that for selected slides, but differs in the following way. A cleaned diatom sample in distilled water is shaken gently and a small amount is pipetted through a large bore onto the centre of a glass slide or cover-slip (No.0. 19 mm diameter) and left to dry, either in a warm 37°C oven or air dried. Drying a strew too quickly (i.e. using a flame) sets up small convection currents and the specimens, especially the smallest, aggregate into a crust at the air/water interface making analysis difficult. Preparation of the strews by drying down overnight before mounting will enable good evenly distributed strews to be made.

This method, if followed carefully, will enable the newcomer to produce diatom (or similar organisms) slides of good quality. Like all skills the initial results may seem poor but with practice ambitious mounts will be achieved.